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The accumulation of plant-produced antimicrobial compounds in response to ectomycorrhizal fungi: a review

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It has been suggested by a number of authors that the accumulation of plant-produced antimicrobial compounds in ectomycorrhizal roots is associated with three phenomena. These compounds may be involved in controlling ingress of compatible mycobionts into feeder roots, they may inhibit colonization of roots by incompatible ectomycorrhizal fungi and their accumulation by ectomycorrhizal roots may be correlated with increased resistance to root pathogens. In addition to assessing these three hypotheses, this paper presents a critical review of the techniques through which they have been derived. The advantages and disadvantages of transmission electron microscopy, optical microscopy, chemical isolation and bioassays to study accumulation of plant-produced antimicrobial compounds in response to ectomycorrhizal fungi are presented.

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Il a été proposé par quelques auteurs que l'accumulation de composés antimicrobiens produits par les racines ectomycorhiziennes soit reliée à trois phénomènes. Ces composés peuvent réduire l'avance des ectomycorhizateurs compatibles dans les racines courtes; ils peuvent bloquer l'infection par les champignons ectomycorhizateurs incompatibles; et leur accumulation dans les racines peut aussi être reliée à la résistance induite aux pathogènes racinaires. En plus de discuter ces trois possibilités, cet article présente une revue critique des techniques employées pour en arriver aux résultats permettant leur formulation. Les avantages et désavantages de la microscopie électronique à transmission, de la microscopie optique, de l'isolation et des essais biologiques de composés chimiques sont présentés en relation avec l'étude de l'accumulation de composés antibiotiques produits par les plantes dans les racines ectomycorhiziennes.

Introduction

The application of ectomycorrhizal fungi to increase forest productivity has been proposed because they enhance nutrient and water uptake, increase resistance to root pathogens and promote the growth of plants in a variety of environmental conditions (Bowen 1973; Hacskaylo 1973; Harley and Smith 1983; Mikola 1973; Peterson *et al.* 1984; Ruehle and Marx 1979).

Numerous reports have discussed the accumulation of phenolics and terpenes in plant roots in response to colonization by ectomycorrhizal fungi. Circumstantial evidence has led several workers to postulate that these compounds are accumulated in response to mycorrhizal fungi in a manner analogous to the accumulation of phytoalexins in a number of host-pathogen interactions.

Phytoalexins are low molecular weight antimicrobial compounds that are both synthesized by, and accumulated in, plants upon exposure to microorganisms (Paxton 1981). Phytoalexin accumulation is associated with disease resistance based on the observation that rapid accumulation of large amounts of phytoalexins occurs in resistant plants whereas in susceptible plants there is either delayed or smaller phytoalexin accumulation in response to pathogenic infections (Deverall 1977; Keen 1981; Kuć 1976; Kuć and Rush 1985; Mansfield 1982, 1983).

It is well documented that phenanthrene phytoalexins participate in controlling fungal ingress and disease resistance in orchid mycorrhizae (Arditti 1979). Consequently, one may hypothesize that phytoalexins are widely involved in mycorrhizal symbioses. This is supported by Morandi *et al.* (1984) who reported the accumulation of isoflavonoid phytoalexins in roots of *Glycine max* cv. Amsoy 71 in response to infection by the vesicular-arbuscular mycorrhizal (VAM) fungi *Glomus mosseae* (Nicol and Gerd.) and *Glomus fasciculatus* (Gerd. et Trappe comb. nov.). Although it is too early to speculate about phytoalexin accumulation in VAM associations in general, the results of Morandi *et al.* (1984) are encouraging.

Although the term phytoalexin has been used to refer to accumulated secondary metabolites in ectomycorrhizal roots, it remains to be demonstrated convincingly in most cases that these compounds qualify as phytoalexins according to the definition given by Paxton (1981). At present, however, there is insufficient information available from ectomycorrhizal systems to permit distinctions to be drawn between metabolites whose synthesis is only enhanced and those whose appearance is tightly coupled to infection, or between those with proven antimicrobial properties and those without. For this reason, we prefer to refer to these compounds as plant-produced antimicrobial compounds.

The accumulation of antimicrobial phenolics and terpenes in response to ectomycorrhizal fungi has been hypothesized to be associated with three phenomena: 1) control of ingress into feeder roots by compatible ectomycorrhizal fungi, 2) prevention of colonization of roots by incompatible ectomycorrhizal fungi, and 3) protection against root pathogens. Although the accumulation of plant-produced antimicrobial compounds in ectomycorrhizal plants has been discussed by many authors, its extent and significance in nature remain controversial because of the use of techniques which are sometimes non-specific and/or inappropriate. The objectives of this paper are twofold. First, we present a critical review of the methods that have been used to investigate the presence of plant-produced antimicrobial compounds in ectomycorrhizal roots. Second, we summarize the most significant studies which have contributed to the hypothesis that these compounds are induced by ectomycorrhizal fungi.

Methods used to investigate the presence of plant-produced antimicrobial compounds in ectomycorrhizal roots

Transmission electron microscopy. Transmission electron microscopy (TEM) of plant samples fixed with osmium tetroxide has suggested to several authors that some of the electron-dense materials observed represent phenolics. However, it is important to note that phenolics are not the sole class of chemicals which react with osmium tetroxide (Meek 1977; Nielson and Griffith 1978). Lipids, and to a smaller extent proteins, also bind this reagent. Moreover, not all phenolics are fixed by osmium tetroxide (Turnbull 1980). Although TEM observations may suggest that phenolics and, perhaps, terpenoids are present in osmium-fixed materials, we believe that localization of some phenolic materials in plant tissues can be carried out more accurately using a number of histochemical techniques and optical microscopy.

Optical microscopy. A wide variety of reagents for detection of phenolics is available, but a general problem is the extreme structural diversity within the phenolic class. It is difficult to find a stain that will indicate unambiguously the presence or absence of phenolics in a given sample. It is, therefore, important that several stains with different specificities be used (Ling-Lee *et al.* 1977). The presence of positively-stained materials after using reagents for phenolic detection, however, cannot by itself confirm the presence of antimicrobial phenolics in plant tissues, since both antimicrobial and non-antimicrobial phenolics may be detected. Similarly, the absence of positively-stained materials does not necessarily indicate the absence of antimicrobial compounds from a sample since some phenolic derivatives, and other antimicrobial compounds such as monoterpenes, may not

be detected by the usual histochemical tests.

General procedure for chemical extraction. Bioassay of plant extracts is necessary in order to determine whether antimicrobial compounds are present in plant tissues. Extraction of low molecular weight phenolics and terpenes can be performed using solvents such as ethanol or methanol for phenolics, and acetone, ether or petroleum ether for terpenes (Harborne 1984). Various extraction procedures can be used, but the investigator must be aware of the presence of artifacts when samples are subjected to excessive heat and light.

The choice of material for extraction is also of critical importance. Since the objective is to determine whether soluble antimicrobial compounds are present at the plant-fungus interface, it is crucial that the samples extracted be free, as far as possible, of tissues that are not relevant to the analysis. The time of sampling must also be optimized since antimicrobial compounds may undergo decomposition or modification *in vivo* either as a result of detoxification by microorganisms or as a result of their high reactivity with their chemical environment (Bailey and Deverall 1971; De Wit and Flach 1979; Duchesne *et al.* 1985, 1986; Higgins 1981; Van Etten *et al.* 1982).

General procedure for bioassay of plant extracts. To test for the presence of antimicrobial compounds in a sample, a wide variety of bioassays is available (Anonymous 1947; Hart 1981; Homans and Fuchs 1970; Smith 1982). It is important that more than one bioassay method be applied to any extract under investigation, as results will vary according to the assay organism and the procedure employed (Hart 1981; Ward *et al.* 1975). It is also imperative that concentrations relevant to *in vivo* concentrations be used for bioassays, and that the assay organisms be the ones against which the test compounds putatively act in nature.

While positive results using bioassays are an indication that antimicrobial compounds are present in the material under investigation, they do not indicate that their occurrence is significant *in situ*. Ideally the activity of antimicrobial compounds should be demonstrated *in situ*, although this is experimentally difficult.

Bioassay of ectomycorrhizal root extracts. Only a few attempts have been made to isolate and identify plant-produced antimicrobial compounds from ectomycorrhizal roots of plants. Extraction of phenolics from ectomycorrhizal roots of *Pinus radiata* D. Don led to the identification of catechin, stilbenes and leucoanthocyanin (Hillis and Ishikura 1969; Hillis *et al.* 1968). Extraction of ectomycorrhizal roots of *Pseudotsuga menziesii* (Mirb.) Franco showed the presence of taxifolin and poriolin (Hillis and Ishikura 1969). Stilbenes have been regarded by many workers as phytoalexins of gymnosperms and angiosperms (Gorham 1980; Hart and Shrimpton 1979; Lyr 1962; Rennerfelt and Nacht 1955; Ward *et al.* 1975). As a result of this, Hillis and Ishikura (1969) postulated that stilbenes acted as fungal inhibitors in ectomycorrhizal roots of *Pinus radiata*. Unfortunately, Hillis and Ishikura (1969) extracted roots from natural soils where the species of mycobiont was unknown. The fungal species is an important parameter governing the accumulation of plant-produced antimicrobial compounds since there is much variation in how species and even races of microorganisms affect secondary metabolism in plants (Deverall 1977; Keen 1981; Kuć 1976; Kuć and Rush 1985; Mansfield 1982, 1983; Stoessl 1982).

After the discovery that up to 80 % of wheat root exudates are comprised of volatiles when seedlings are grown on acidic substrates (McDougall 1970), attempts were made to monitor the synthesis of volatile organic compounds by ectomycorrhizal root systems of *Pinus sylvestris* L. after inoculation with *Boletus variegatus* Fr. (Krupa and Fries 1971). The compounds, 3-carene, α-pinene, and terpinolene were the most abundant constituents and showed a two- to eight-fold increase in 3-month-old ectomycorrhizal root systems compared to control roots.

The same paper reports that phenolic accumulation is also induced in ectomycorrhizal root systems, but the identity of the phenolics is not stated.

Bioassays showed that radial mycelium growth of the ectomycorrhizal fungi, *Boletus variegatus* and *Rhizopogon roseolus* (Cora) Th. Fr. was inhibited by small amounts of 3-carene, α-pinene, terpinolene and of other terpenes (Melin and Krupa 1971). The same substances were also fungistatic, although to a smaller extent, to the root pathogenic fungi *Phytophthora cinnamomi* Rands and *Fomes annosus* Fr. (Krupa and Nylund 1972). No absolute quantification of the fungitoxic terpenes from ectomycorrhizal root systems has been carried out, however, which renders interpretation of these results difficult. Inoculation of *Pinus echinata* Mill. with *Pisolithus tinctorius* (Pers.) Coker and Couch resulted in an approximate 40-fold increase in the level of 3-carene whereas inoculation with *Cenococcum graniforme* (Sow.) Ferd. and Winge induced an approximately 30-fold increase in β-phellandrene four months after inoculation (Krupa *et al.* 1973). Unfortunately, no attempt was made to quantify terpenes earlier after inoculation (Krupa and Fries 1971; Krupa *et al.* 1973). Sohn (1981) attempted to study *in vitro* toxicity of phenolics extracted from ectomycorrhizal roots of *Pinus taeda* L. inoculated with *Pisolithus tinctorius*. Unidentified phenolics produced by the host plant inhibited germination of *Fusarium oxysporum* Schlecht. emend Snyd & Hans *in vitro*.

Phenylpropanoid enzymes from ectomycorrhizal roots. More recently, attempts have been made to determine whether enzymes that are associated with the biosynthesis of phenylpropanoid phytoalexins and lignin are induced in the ectomycorrhizal association between *Pinus sylvestris* and the compatible ectomycorrhizal fungus *Laccaria laccata* (Scop. ex Fr.) Berk. and Br. Ronald and Söderhäll (1985) compared phenylalanine ammonia lyase (PAL) and peroxidase activities in mycorrhizal and control roots of *Pinus sylvestris* 14 weeks after inoculation. No significant difference in either PAL or peroxidase activities was observed. In addition, PAL and peroxidase activities failed to be induced by known elicitors of these enzymes. Subsequently, the authors (Ronald and Söderhäll 1985) concluded that phenolic accumulation has little or no significance in ectomycorrhiza formation in *Pinus sylvestris*. These results disagree with those of Krupa and Fries (1971), who reported that phenolic accumulation is enhanced in ectomycorrhizal root systems of *Pinus sylvestris* after inoculation with *Boletus variegatus*. It is not possible, however, to determine if this apparent contradiction is caused by the different mycobionts that were used in these two experiments or if the regulation of the phenylpropanoid pathway in *Pinus* operates elsewhere than at the PAL level.

Accumulation of plant-produced antimicrobial compounds and control of fungal ingress

Control of the extent of root colonization by ectomycorrhizal fungi has long been a source of speculation. It appears that plant defense reactions are initiated which prevent the fungus from reaching the vascular system. As early as 1944, MacDougal and Dufrénoy reported the accumulation of tannin-like substances in mycorrhizal roots. They postulated that these compounds prevent ectomycorrhizal symbionts from invading the stele.

Foster and Marks (1966) made a significant contribution to this field when they reported the presence of a tannin layer consisting of tanniferous cells in the outer cortex of ectomycorrhizal roots of *Pinus radiata*. This layer was described as a layer of cells distinguished by the high electron density in the cell vacuolar contents. Although there was no direct evidence for a fungitoxic or fungistatic effect of the tannin layer on penetrating hyphae, Foster and Marks proposed that the tannin layer controls the morphogenesis of the Hartig net. This proposal was supported by the observation that fungal hyphae become distorted in areas that are proximal to the tannin-filled cells, suggesting that the putative tannin content of these cells has fungitoxic or fungistatic effects on the penetrating

hyphae of ectomycorrhizal fungi (Foster and Marks 1966, 1967). Consequently, it was proposed that Hartig net formation through development of a labyrinthine network of hyphae is the direct result of fungitoxic chemicals produced by the plant. However, Piché *et al.* (1983) could not find a correlation between Hartig net formation and the penetration of the tannin layer described by Foster and Marks.

The tannin layer, furthermore, was not demonstrated to be fungitoxic since no extraction and bioassay of the tannin layer had been carried out. Moreover, tannins have also been found in uninfected roots of several species of gymnosperms and angiosperms (Bonfante-Fasolo and Scannerini 1977; Duddridge and Read 1984a, 1984b; Marks and Foster 1973; Nylund 1981; Piché *et al.* 1981).

Piché *et al.* (1981) described the first time course study on phenolic accumulation in response to ectomycorrhizal infection. Observation with optical microscopy suggested the presence of phenolics but failed to show quantitative differences between ectomycorrhizal and control feeder roots of *Pinus strobus* L. 1, 5, 12, and 23 days after inoculation with *Pisolithus tinctorius*.

Contrasting results have been reported from another ectomycorrhizal system. Using a battery of histochemical tests to localize phenolics in ectomycorrhizal roots of *Eucalyptus fastigata* Deane and Maiden, Ling-Lee *et al.* (1977) have demonstrated that ectomycorrhiza formation triggers accumulation of phenolics in the root epidermis but not in other root tissues. Since this work was carried out on ethanol-dehydrated tissues, the phenolics that were detected had little solubility in ethanol, and it is likely that the soluble phenols had been lost during tissue preparation. The results presented by Ling-Lee *et al.* (1977) illustrate the superiority of histochemical techniques and light microscopy over TEM of osmium-fixed materials, in localizing certain phenolic substances.

Accumulation of plant-produced antimicrobial compounds and compatibility

Specificity in ectomycorrhizae. Ectomycorrhizal fungi show different degrees of specificity for their host plant. Molina and Trappe (1982) classified ectomycorrhizal fungi into three classes of specificity. Fungi such as *Laccaria laccata*, *Pisolithus tinctorius* and *Paxillus involutus* (Batsch.) Fr. show little host specificity because they can colonize a large number of plant species; fungi such as *Alpova diplophloeus* (Zeller and Dodge) Trappe and Smith and *Rhizopogon vinicolor* A.D. Smith show specificity within one genus; and the third class includes those fungi with intermediate degrees of specificity for their host. Reciprocally, plants also show signs of specificity for their ectomycorrhizal symbionts. This is well illustrated by Navratil (1986) who reported that different seed sources of *Picea glauca* (Moench.) Voss and *Pinus contorta* Douglas show different degrees of colonization by the same strains of ectomycorrhizal fungi. Results published by Marx and Bryan (1971) corroborate these observations.

Compatibility in ectomycorrhizal associations. Unlike compatibility in host-pathogen interactions, compatibility in mycorrhizal fungus-plant interactions leads to a beneficial effect on the plants (Harley and Smith 1983). Several factors are regarded as potential determinants of plant-fungus compatibility in ectomycorrhizal symbioses. Among these are the production of siderophores (Szaniszlo *et al.* 1981) and antibiotics by ectomycorrhizal fungi (Marx 1973; Zak 1964); selective stimulation of desirable fungi by plant root exudates (Birraux and Fries 1981; Fries 1981; Fries and Birraux 1980; Fries *et al.* 1985); enhanced peroxidase activity and change in lignification at the root surface (Anderson 1985). Much research has yet to be carried out to demonstrate whether these putative determinant phenomena are either significant and/or ubiquitous in nature. It has been postulated that incompatible in-

teractions between ectomycorrhizal fungi and plants is associated with the accumulation of plant-produced antimicrobial compounds (Anderson 1985).

Compatibility and hypersensitive reactions. Molina (1981) observed that *Paxillus involutus* induces a plant reaction on *Alnus* sp. that resembles hypersensitivity, the rapid necrosis of cells in response to stress agents. Epidermal and cortical cells directly in contact with the mantle hyphae were deeply safranin-stained, apparently due to the deposition of polyphenolic materials in the cell walls. No such reaction is induced in compatible ectomycorrhizal interactions. Moreover, Molina (1981) also reported quantitative differences between reactions triggered by *Paxillus involutus* and those induced by other incompatible ectomycorrhizal fungi suggesting that there may be variation in the degree of incompatibility between plants and ectomycorrhizal fungi. It was not determined whether the polyphenolic materials that were accumulated in these incompatible interactions were fungitoxic, but the extent of colonization of feeder roots by incompatible ectomycorrhizal fungi was apparently limited by the accumulation of polyphenolic materials (Molina 1981). Because Molina's (1981) observations were carried out four months after inoculation of the seedlings with the fungus, it is not known if the deposition of polyphenolic materials and the exclusion of the fungus were related phenomena. Nevertheless, observations from several other plant-ectomycorrhizal fungus interactions suggest that phenolic accumulation is associated with hypersensitive incompatible interactions in both gymnosperms and angiosperms (Malajczuk *et al.* 1982, 1984; Molina and Trappe 1982).

Compatibility and tolerance to plant-produced antimicrobial compounds. It has been postulated by many authors (Hillis and Ishikura 1969; Nylund 1981; Piché 1982) that the presence of plant-produced antimicrobial compounds in ectomycorrhizal roots could selectively inhibit the growth of undesirable microorganisms. However, bioassay of terpenes from *Pinus sylvestris* showed that two ectomycorrhizal fungi were more sensitive to the compounds than were root pathogenic fungi (Melin and Krupa 1971). Thus, one may not assume that specificity in ectomycorrhiza formation is uniquely associated with tolerance to the antimicrobial compounds produced by the host plant.

Compatibility and fungal elicitors. Coleman and Anderson (1985a, 1985b) studied the elicitation of phenolics in callus cultures of *Pinus contorta* and *Pseudotsuga menziesii* by elicitor preparations from the culture filtrates of *Rhizopogon vinicolor* and *Rhizopogon occidentalis* Zeller and Dodge. *Rhizopogon occidentalis* is specific to *Pseudotsuga menziesii* whereas *Rhizopogon vinicolor* is specific to *Pinus contorta* (Molina and Trappe 1982). Coleman and Anderson's results (1985a, 1985b) indicated that both fungi can induce phenolic accumulation in the callus cultures of these two species regardless of their host specificity. These results were obtained, however, by using general spectrometric procedures for phenolic determination. Consequently, it is not known whether individual chemical species show the same accumulation pattern in response to compatible and incompatible ectomycorrhizal fungi.

Accumulation of plant-produced antimicrobial compounds and resistance to root pathogens

The protective influence of ectomycorrhizal fungi against root pathogens has been known for a long time (Marx 1973; Perrin 1985a, 1985b; Zak 1964). Several hypotheses have been advanced to explain this phenomenon (Zak 1964). Production of antibiotics by ectomycorrhizal fungi and accumulation of plant-produced antimicrobial compounds by ectomycorrhizal roots are the two most plausible explanations. Although production of antibiotics in pure cultures of ectomycorrhizal fungi has been widely documented (Marx 1973), it is generally admitted that antibiosis alone cannot account for induced resistance in all of the ectomycorrhizal associations that have been studied (Harley and Smith 1983).

Antibiosis and disease resistance. *Fusarium oxysporum* f. sp. *pini* is a root pathogen of conifers which infects mainly primary roots in a six-week period following seed germination, although symptoms may appear later during seedling development (Bloomberg 1973). It was determined that ectomycorrhizal seedlings of *Pseudotsuga menziesii* show greater resistance to *Fusarium oxysporum* than control seedlings inoculated with the pathogen alone (Sampangi *et al.* 1985; Sinclair *et al.* 1975, 1982; Stack and Sinclair 1975). Despite the fact that *Laccaria laccata* shows antagonism to *Fusarium oxysporum in vitro* (Sylvia and Sinclair 1983a) it is doubtful that this mycorrhizal fungus produces sufficient amounts of antibiotics to account for enhanced resistance of *Pseudotsuga menziesii* to *Fusarium oxysporum in vivo* (Sylvia and Sinclair 1983a). Moreover, it was shown that the presence of *Laccaria laccata* could also deter root rot in *Pseudotsuga menziesii* even when the ectomycorrhizal fungus was separated from the plant by a dialysis membrane. The influence of *Laccaria laccata* on root disease suppression is not associated with a physical barrier effect such as the one described by Marx and Davey (1969) with *Pisolithus tinctorius*.

Phenolic induction by *Laccaria laccata*. Sylvia and Sinclair (1983b) have shown histochemically that *Laccaria laccata* induces the accumulation of large quantities of phenolic compounds in primary roots of *Pseudotsuga menziesii*. In contrast, inoculation with *Fusarium oxysporum* led to the accumulation of small amounts of phenolics. Inoculation with known root protectant organisms such as *Pseudomonas ceparia* Burkh. and *Trichoderma harzianum* Rifai induced the same levels of phenolics as those induced by *Laccaria laccata*. Because neither significant antibiosis nor physical barrier effects by *Laccaria laccata* could be demonstrated, it was postulated that the interaction between *Laccaria laccata* and primary roots of *Pseudotsuga menziesii* induces resistance to *Fusarium oxysporum* through the accumulation of fungitoxic phenolics in the roots. Attempts are presently being made at Cornell University to determine the chemical nature of those compounds (W.A. Sinclair, personal communication). In addition, the lack of induction of phenolic accumulation observed in roots of *Pseudotsuga menziesii* inoculated with *Fusarium oxysporum* suggests that this fungus exerts a suppressive effect on phenolic accumulation. This is consistent with observations from several host-pathogen interactions (Bushnell and Rowell 1981; Heath 1981, 1982; Tepper and Anderson 1984).

Similar experiments have been carried out on the protection of *Picea abies* (L.) Karst against *Fusarium* root rot. Induced resistance to *Fusarium oxysporum* has been correlated with increased levels of the terpenes, limonene, myrcene and terpinolene in ectomycorrhizal feeder roots (Sampangi and Perrin 1985). Although these chemicals show antimicrobial activity *in vitro* (Melin and Krupa 1971) the interpretation of Sampangi and Perrin's results is uncertain since no quantification of the fungitoxic materials has been reported. Nevertheless, it is noteworthy to mention that the volatile nature of these compounds may be linked to dramatic decreases of the *Fusarium oxysporum* populations in soils inoculated with *Laccaria laccata*.

Conclusion

In this paper we have presented what appears to be the most significant results concerning the accumulation of plant-produced antimicrobial compounds in ectomycorrhizae. Although numerous studies have dealt with this phenomenon, there are no reports which undisputably indicate that plant-produced antimicrobial compounds are responsible for either the control of fungal growth, the exclusion of incompatible ectomycorrhizal fungi or disease resistance. Sufficient circumstantial evidence is available, however, to allow us to postulate that plant-produced antimicrobial compounds are indeed associated with these three phenomena, although their significance is still not clear. Considering the intimacy of the interactions between plant root tissues and their ectomycorrhizal symbionts, it is very likely that many molecular mechanisms are involved in the recognition of compatible partners and the development of fully functional ectomycor-

rhizal symbiosis. Production of antimicrobial metabolites by the plant, and the response of fungal symbionts to their presence, will probably be one facet of this complex process.

It appears that both compatible and incompatible ectomycorrhizal associations trigger the accumulation of plant-produced antimicrobial compounds. The results published by Molina (1981) and Malajczuk *et al.* (1984) indicate that the accumulation of plant-produced antimicrobial compounds may be greater in incompatible interactions than in compatible interactions. Moreover, it seems that different levels of compatibility or incompatibility exist within both groups. Before these observations can be evaluated, however, it would be important to compare the kinetics of antimicrobial accumulation in compatible and incompatible interactions. If it is true that incompatible interactions lead to greater accumulation of plant-produced antimicrobial compounds than compatible interactions, one may postulate that protection against root pathogenic fungi should be greater when plants are inoculated with incompatible ectomycorrhizal fungi.This phenomenon and its possible applications are under investigation in our laboratories.

Genetic manipulation of ectomycorrhizal fungi has been proposed as a means of increasing the beneficial effects of mycorrhizal symbioses (Hacskaylo 1983; Hirsch 1984; Peterson *et al.* 1984). The availability of recombinant DNA technology should also make it possible to construct plant symbiotic microorganisms displaying economically advantageous characters borrowed from the ectomycorrhizal symbiosis. Such microorganisms might or might not be mycorrhizal, they would ideally be easy to grow in pure culture and they should be adapted to the range of environmental conditions encountered in natural forest ecosystems. These biotechnological approaches have yet to be applied successfully because of a lack of knowledge of the molecular mechanisms which govern the establishment and maintenance of ectomycorrhizal symbioses (Gianinazzi-Pearson 1984; Peterson *et al.* 1984; Ruehle and Marx 1979). Understanding the nature and role of plant-produced antimicrobial compounds in ectomycorrhizal formation should eventually benefit those forest biotechnologists interested in utilizing ectomycorrhizae native or modified to increase forest productivity.

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